## REMARKS

Upon entry of the present amendment claims 1, 5, 7-11, 14, 31-32 will be pending in the application. Claims 1, 11, and 14 were amended for clarity as per Examiner's suggestion. Claims 2 and 3 (previously dependent on claim 1) were canceled and are now presented as independent claims 31 and 32. No new matter was added.

Reconsideration of the application is respectfully requested.

***Claim Objections***:

Claims 1, 11 and 14 stand objected on the following grounds:

Claim 1 is objected to for lack of consistency with respect to recitation of the term "eukaryotic," which term appears in parts (a)-(b) but not in the preamble. As per the Examiner's suggestion, Applicants have now amended claim 1 to recite "eukaryotic" in the preamble.

Claims 11 and 14 are objected to for reciting "substrate selected from" rather than "substrate selected from the group consisting of." As per the Examiner's suggestion, Applicants have now amended claims 11 and 14 to recite "group consisting of."

In view of the above amendments, withdrawal of the objections is respectfully requested.

***Rejections under 35 U.S.C. 112, 2nd paragraph***

Claims 2, 3, and 14 are rejected as being indefinite on the following grounds:

Claim 14 is rejected for depending on a canceled claim. Claim 14 have been amended to depend from claim 1.

Claims 2 and 3 are rejected for recitation of "ST3Gal 3 protein is truncated," and "unpaired cysteine in the ST3Gal3 protein is removed by substitution with a non-cysteine residue," respectively. Since the Examiner has treated claims 2 and 3, for purposes of examination, as independent claims, claims 2 and 3 were re-written in independent format and are now presented as "newly added" claims 31 and 32. Claim 31 recites subject matter previously recited in claims 1 and

2, and claim 32 recites subject matter previously recited in claims 1 and 3. Thus, no new matter was added.

In view of the above amendments, withdrawal of the rejections is respectfully requested.

***Rejections under 35 U.S.C. Section 103a***

***(a) Rejection of claims 1-2, 7, 10-11 and 14***

At the outset, Applicants point out that in view of the amendment of claim 2 (discussed above) and its presentation in independent format as claims 31, the rejection of claim 2 is now addressed with respect to newly-added claim 31.

Claims 1-2(31), 7, 10-11 and 14 remain rejected as unpatentable over Paulson *et al.* (US 5,858,751) in view of Hellman *et al.* (1995) and Clark *et al.* (2001) on the following grounds:

(i) Paulson *et al.* teach methods and expression of eukaryotic α(2,3) sialyltransferase (ST3Gal3) proteins in prokaryotic organisms;

(ii) Clark *et al.* teach methods of isolation, purification and refolding of insoluble protein from inclusion bodies using redox buffers; and

(iii) Hellman *et al.* teach solubilizing insoluble protein from inclusion bodies using protein fused to maltose binding domain (MBD).

The Examiner maintains that it would have been obvious to combine the teachings of the cited references because they "provide not only the individual elements of the claimed method but also motivation to combine these elements as well as a reasonable expectation of success" of arriving at the methods recited in Applicants' present claims. [Office Action mailed August 4, 2009, par. spanning p. 8- 9]. Applicants respectfully traverse the rejection.

Applicants submit that according to the present claims, a recombinant eukaryotic α(2,3) sialyltransferase (ST3Gal3) protein comprising a maltose-binding domain is produced (i) starting with an insoluble protein (*e.g.*, in bacterial inclusion bodies), (ii) is refolded outside of a cellular environment (*e.g.,* using a redox couple buffer), and (iii) is catalytically active (*e.g.*, in transferring

sialic acid sugar from a donor substrate to an acceptor substrate). Applicants respectfully submit that even if (for purposes of this argument) a person of skill in the art would have had a reason to try out the teachings of the cited art in an attempt to produce eukaryotic ST3Gal3 of Paulson using methods of Clark and Hellman, s/he would not have had reasonable expectation of success that an ST3Gal3 protein that was solubilized, refolded *and* active as required by the present claims, could have been produced. This is because the references at best teach general approaches based on which the methods recited in the present claims would not have been predictable. This is discussed in detail in the following paragraphs.

First, in their Response filed on July 2, 2009, Applicants had discussed in detail the relevance of the recent US Supreme Court's decision, *KSR International Co. v. Telefex Inc.*, 550 U.S. _, 82 U.S.P.Q.2d 1385 (2007), and subsequent Federal Circuit Court's decisions dealing with inventions in life sciences arts, to the present context.

However, there is no indication in the Official Action of August 4, 2009, that the claims were examined, as they must be, following the above mentioned precedents. Specifically (as shown in more detail bellow), the Examiner relies on the "general approach that seemed to be a promising field of experimentation" (*see Pfizer v Apotex*, 480 F.3d 1348 (Fed. Cir. 2007) to support the statements of reasonable expectation of success. This is not a proper standard, and the Office has failed to meet the *prima facie* case of obviousness.

For convenience, certain relevant legal points are summarized below:

(1) In *KSR,* the Supreme Court stated that "...the Federal Circuit had erred by applying the teaching-suggestion-motivation (TSM) test in an overly rigid and formalistic way." Importantly, the Supreme Court recognized (and affirmed years of jurisprudence) that predictability was a necessary component of obviousness analysis;

(2) Subsequently, Federal Circuit Court applied the holding of *KSR* (which dealt with a mechanical invention) to less predictable, life sciences inventions. *E.g. Eisai Co. v. Dr. Reddy's Labs.,* 533 F.3d 1353 (Fed. Cir. 2008). In *Eisai,* the Court stated that chemical arts are often unpredictable and "KSR's focus on

these 'identified, predictable solutions' may present a difficult hurdle because potential solutions are less likely to be genuinely predictable." *See also, Sanofi-Synthelabo v. Apotex, Inc.*, 550 F.3d 1075 (Fed. Cir. 2008) (holding that disclosure of the racemic mixture did not make the isolated racemate *prima facie* obvious); *P&G v. Teva Pharms. USA,* 2009 U.S. App. LEXIS 10475 (Fed. Cir. 2009) (holding that a positional isomer was not obvious over the prior art compound).

In short, for life sciences technologies, even seemingly "minor" differences between the invention and the prior art were held unpredictable. In the present case, due to the nature of the art, and the evidence presented herein and of record, the invention recited in the present claims *would not have been predictable* from the combined teachings of cited references as shown below.

With respect to the cited references, Applicants note the following and address the Examiner's concerns made in the most recent Official Action (under "Arguments and Response;" pages 7-12) [hereinafter "OA of 8/4/09"]:

Paulson discloses production of recombinant sialyltransferases in a eukaryotic expression systems (*e.g.* monkey kidney cells (COS), insect cells (Baculovirus system)) (*see*, Paulson, *e.g.*, Example 3, 6, 7, 13). With respect to prokaryotes, Paulson mentions that recombinant proteins can be produced in prokaryotic expression systems (*see*, Paulson, column 11, lines 25-66), and also states generically that "alternatively, unglycosylated sialytransferase is produced in recombinant prokaryotic cell culture." (*see*, Paulson, column 10, lines 38-40, emphasis added). But, this disclosure is deficient because a person of skill in the art reading Paulson could not have learnt whether (i) the unglycosylated protein Paulson mentions was in fact produced, and if so (ii) it was, or could have been, produced in soluble form, and (iii) it could have been refolded in the active form as recited in Applicants' present claims.

The Examiner has addressed point (i) above acknowledging that Paulson did not provide any working examples but stated that there was no reason to doubt that the enzyme could have been produced in prokaryotic systems (OA of 8/4/09, page 9, 1st par.). The Examiner then relies on Clark and Hellman to remedy Paulson's deficiencies regarding points (ii) and (iii) above.

The Examiner states that "there is nothing unpredictable or unexpected from obtaining an active enzyme from inclusion bodies [since] [m]any proteins and enzymes have been over expressed and purified from inclusion bodies" [OA of 8/4/09, page 8, 2nd par.]. However, this broad, conclusory statement is supported by Hellman alone, which discusses refolding of one enzyme (cyclomaltodextrin glucanotranferase). *Id.* Additionally, the Examiner states that "[i]t is well known in prior art, how to produce biologically active soluble eukaryotic proteins by expressing in a prokaryotic organism by fusion of the protein with the MBD tag, solubilize from the inclusion bodies and refold using refolding buffer" [OA of 8/4/09, page 8, 2nd par. citing Hellman, Bach and Kapust]. Again, the Examiner supports this broad statement by relying on results of a single enzyme (Hellman) and on the art that is not even relevant—Bach states that it accomplished "efficient functional expression [of scFvs] in the cell cytoplasm in a soluble, active form" (*see* Abstract, emphasis added); and Kapust uses MBP fusion to promote expression of soluble proteins in the cytoplasm (*see* entire article, e.g. Methods section, page 1673, 2nd col. 2nd full par.)—Applicants claims recite refolding outside of the cell. The Examiner has failed to establish that the techniques recited in the above mentioned references are routine to apply to any protein and irrespective of whether the refolding is performed within or outside of the cell. Thus, no *prima facie* case of obviousness was met.

In fact, the Examiner could not have met the *prima facie* case of obviousness since evidence of unpredictability existed in the art. Arriving at the present invention is not as straightforward as the Examiner appears to believe.

For example, comparing the results of Kapust (mentioned above) and the present specification, it is evident that the effect of MBP fusion on solubility and proper folding is protein specific. Kapust shows that certain proteins (e.g. TEV protease) can be expressed in soluble form in the cytoplasm of *E. coli* when fused to MBP (without MBP fusion, TEV protease was not soluble) (Kapust, page 1669, col. 2, last par. – 1671, col. 1, 1st par.). If all proteins were to behave the same way when fused to MBP, as the Examiner believes, ST3GalIII enzyme recited in Applicants' claims would have been produced in soluble and properly refolded form inside of the *E. coli* cell and there would have been no need to purify the inclusion bodies and experiment to arrive at the present invention (*see* specification examples, page 76-78, describing purification of inclusion bodies, solubilization and refolding). In fact, Kapust states that "it is not known whether the solubility of

many different polypeptides can be improved by fusing them to a highly soluble partner or whether this approach is only effective in a small fraction of cases." (Kapust, page 1668, col 2).

As further illustrated in the attached publication, the fact that "a protein or protein domain is produced soluble when fused to ….a carrier protein *does not guarantee* that the protein of interest is properly folded *and* active." (*see*, Attachment 1: Nomine Y. *et al.*, Formation of soluble inclusion bodies by HPV E6 oncoprotein fused to maltose-binding protein, Protein Expression and Purification, 23, 22-32 (2001), page 22, column 1, emphasis added). Nomine showed that most of the E6 moieties even in his *soluble* MBP-E6 preparations were *misfolded* and *unable* to interact with a biologically active ligand (*see*, Nomine, page 30, paragraph spanning col. 1 and 2). Thus, based on the knowledge in the art, a person of skill in the art could not have predicted what the effect, if any, of MBD fusion would have been on protein solubility and refolding, let alone its activity. In other words, from the cited art, a skilled individual would not have known whether preparing an MBD fusion of a specific type of protein, *e.g.*, ST3GalIII recited in Applicants' present claims, would have had any effect (adverse or beneficial). Applicants arrived at this understanding based on their experimentation.

To further emphasize the need for experimenting with general strategies (as those in Clark), and strategies designed for specific proteins (as those in Hellman), before arriving at a soluble and properly folded protein to achieve functional recombinant protein of a specific type (here ST3GalIII), Applicants attach a publication which discusses challenges of applying general methodologies to a specific enzyme, a glycosyltransferase (*see,* Attachment 2- Boeggemann E. E., *et al.*, The N-terminal stem region of bovine and human β1,4-galactosyltransferase I increases the *in vitro* folding efficiency of their catalytic domain from inclusion bodies, Protein Expression and Purification 30 (2003) 219-229)). Boeggeman worked with inclusion bodes of the protein and underscored the distinction between protein solubility, folding, and activity. Thus, a skilled individual reading Boeggemann would have been cautioned against arriving at a conclusion with respect to one of the above parameters based on the other (*i.e.*, one is not predictive of the other(s)). Based on her studies, Boeggemann concluded that "a protein domain that is produced in a soluble form *does not guarantee* the presence of the protein molecules in a properly folded *and* active

form." (*see,* Boeggemann, abstract, emphasis added). Thus, experimentation is needed on a case-by-case basis as taught by the cited art,[1] and the above mentioned supporting evidence.

In summary, the combination of cited references, at best, merely suggests pursuing a "general approach that seemed to be a promising field of experimentation" (*see Pfizer v Apotex*, 480 F.3d 1348 (Fed. Cir. 2007) citing *In re O'Farrell*, 853 F. 2d 894, 903 (Fed. Cir. 1988)). This is not a standard for obviousness.

*Unexpected Benefits*

Applicants' claims are patentable for an additional reason—unexpected benefits are associated with the claimed invention.

In their Response filed July 2, 2009, Applicants had discussed their unexpected benefit findings with respect to MBP-ST3GalIII. However, in the most recent rejection, the Examiner states that "Applicants have provided no evidence or a reasonable scientific argument to support … unexpected results" [OA of 8/4/09, page 11, last par.]. The Examiner has failed to discuss or explain why the unexpected benefits were not addressed. It is well established, under the US Patent Law, that the "unexpected benefits" must be considered (when present) as part of the obviousness analysis. Moreover, "unexpected benefits" must be considered even if they are not explicitly recited in the claims.

Applicants unexpectedly discovered that "MBP domains can enhance refolding of insoluble eukaryotic glycosyltransferases after solubilization of the proteins from *e.g.*, an inclusion body" (*see*, specification, page 36, lines 13-15). This is in contrast to the cited art, Hellman, that observed that MBD fusion to ssCGT "did not significantly affect the in vitro folding." (see, Hellman, page 56, abstract, last sentence, emphasis added). Applicants have also shown that "refolded MBP-ST3GalIII enzymes were *more active* in transfer of sialic acid to a glycoprotein acceptor molecule" than refolded GST-ST3GalIII (*see*, specification, page 76, lines 21-22, emphasis added). These benefits were not suggested by the prior art.

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[1] Clark acknowledges the numerous challenges encountered in the recovery of active proteins from insoluble inclusion bodies, and admits in his conclusion that "optimum conditions have to be determined on a *case by case* basis." (*see*, Clark, entire text and p. 206, conclusions section, emphasis added).

Therefore, in view of the nature of the art, and evidence presented herein and of record, a person of skill in the art would not have been able to reasonably predict the outcome of following general approaches of Paulson, Hellman and Clark for MBD-ST3GalIII protein. Moreover, as discussed above, the methods recited in pending claims have the unexpected benefit of enhanced refolding and producing a more active enzyme in comparison to other approaches.

Withdrawal of the rejection is respectfully requested.

***(b) Rejection of claims 3(32) and 5***

At the outset, Applicants point out that in view of the amendment of claim 3 and its presentation in independent format as claims 32, the rejection of claim 3 is now addressed with respect to newly-added claim 32.

Claims 3 (32) and 5 stands rejected as obvious over the combination of references cited above and further in view of Ramakrishnan *et al.* (2001) on the above-mentioned grounds and further on the ground that Ramakrishnan teaches that "mutation of unpaired cysteine, Cys 342 to Thr of a beta-galactosyltransferase resulted in 2 to 3 fold increase in yield of refolded enzyme" [Office Action, page 12, 3rd full par.]. Applicants respectfully traverse the rejection.

Claims 3 (32) and 5 are patentable over the prior art for the reasons, and in view of evidence, presented above and further because Ramakrishnan, who worked with a protein different from MBD-α(2,3) sialyltransferase (ST3Gal3), fails to provide any additional or specific guidance to arrive at the present invention. Without more, a person of skill in the art would not have been able to reasonably predict the outcome of following approaches of the cited art.

Moreover, the Examiner's statement that "whether the substitution of the cysteine residues increases refolding yield or not is irrelevant" because claims do not contain a limitation to that effect (OA of 8/4/09) is not supported by the US Patent Law—it is well established that "unexpected benefits" need not be recited in the claims. Reconsideration is in order.

Withdrawal of the rejection is respectfully requested.

*(c) Rejection of claim 5*

Claim 5 stands rejected as obvious over Paulson *et al.* in view of Hellman *et al.* and Clark *et al.* and further in view of Nilsson *et al.* on the grounds mentioned above and further on the ground that Nilsson *et al.* teach the use of affinity tags (*e.g.,* glutathione-S-transferase (GST), poly-His domains) for purification of proteins using affinity columns. Applicants respectfully traverse the rejection.

In view of the fact that Nilsson is a general review of affinity fusion strategies and does not disclose or suggest MBD-α(2,3) sialyltransferase (ST3Gal3) protein or protein refolding strategies, Applicants submit that Nilsson's disclosure does nothing to remedy the deficiencies of the references discussed above. Thus, for the reasons, and in view of evidence, presented above Nilsson in combination with Paulson, Hellman and Clark fails to provide reasonable expectation of success at arriving at the invention of claim 5.

Withdrawal of the rejection is respectfully requested.

*(c) Rejection of claims 8 and 9*

Claims 8-9 stand rejected as obvious over Paulson *et al.* in view of Hellman *et al.* and Clark *et al.* on the grounds mentioned above, and on the ground that Paulson teaches that "such sialytransferases may be employed in multienzyme systems" (*see,* Paulson, column 4, line 28-30). Applicants respectfully traverse the rejection.

Applicants submit that Paulson's disclosure of multienzyme systems is in the context of their function for "synthesis of oligosaccharides and their derivatives." (*see,* Paulson, column 4, line 30), and not in the context of refolding multiple soluble enzymes in one reaction mixture. While use of multienzyme systems may suggest desirability of producing active enzymes together in one reaction mixture, a person of skill in the art would have had, for the reasons and evidence presented above, even less expectation of success than with a single enzyme. Withdrawal of the rejection is respectfully requested.

**Conclusion**

In view of the above amendments, remarks and evidence, Applicants believe that the application is in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

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